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Effect of a Modified Nd:YAG Laser Technique on Neuroma Formation: An Experimental Study in Rat Sciatic Nerve

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Background and Objectives: **Traumatic transection of a peripheral nerve is inherently associated with the development of neuroma at the end of the proximal stump, often leading to therapy-resistant pain. This study was designed to evaluate whether the neodymium:yttrium aluminum garnet (Nd:YAG) laser could prevent neuroma formation after neurectomy.**
Study Design/Materials and Methods: **The sciatic nerves of 14 rats were diffuse coagulated by defocused Nd:YAG laser (12 W power), and subsequently transected with additional focused laser energy. The control group consisted of contralateral nerves transected by microscissors. The nerves were reexposed at different time intervals up to 9 weeks after surgery, and evaluation consisted of macroscopy, and light and transmission electron microscopy.**
Results: **True neuroma formation could not be observed after laser transection, and only five nerves formed a neuromatous bulb, with minimal adhesions to surrounding tissue. Microscissor transection resulted in widespread amputation neuromas, consisting of regenerating axons and connective tissue, and nervous tissue regenerating into surrounding tissue. Laser-transected nerves showed degenerative changes of the axons and myelin, while proliferation of Schwann cells could not be observed. No outgrowth of axons could be observed outside the coagulated proximal stump. An epi/perineurial layer was present, covering the nerve stumps. Microscissor-transected nerves showed proliferation of fibroblasts and Schwann cells, forming minifascicles, and vigorous outgrowth of axons into the tissue and even into the distal nerve stump.**
Conclusions: **Within the limitations of this study it is concluded that the formation of amputation neuromas is suppressed by Nd:YAG laser application by thermal coagulation of the nerve and suppression of Schwann-cell proliferation. Lasers Surg. Med. 25:213–218, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: histology; laser surgery; neuroma prevention; peripheral nerve; traumatic neuroma

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INTRODUCTION

Traumatic transection of a peripheral nerve is inherently associated with the development of a neuroma on the end of the proximal stump. This posttraumatic neuroma consists of regenerating nerve fibers intermingled with connective tissue [1], and often gives rise to therapy-resistant pain [2]. Various methods have been proposed for limiting axonal growth at the proximal stump of a transected peripheral nerve, but none of these techniques have provided satisfactory results to inhibit neuronal growth. Over the years, the use of the CO_2 laser for prevention of neuromas has attracted interest [3–5]. However, studies of CO_2 laser neurectomy have shown controversial results. It has been suggested that the neodymium:yttrium aluminum garnet (Nd:YAG) laser may be more suitable for preventing neuroma formation due to its deeper light penetration in tissue [6].

As a consequence, this study was conducted to study the histological events and neuroma formation in the rat sciatic nerve after a modified Nd:YAG laser neurectomy.

MATERIALS AND METHODS

In total, 14 female rats of an inbred Wistar strain were used in the experiments. The rats (250–300 g) were housed, six at most, in a cage and were kept before the experiments under conventional laboratory conditions. Before surgery, general anesthesia was accomplished by intraperitoneal injection of ketamine (90 mg/kg), xylazine (10 mg/kg), and atropine (0.05 mg/kg) mixture.

Surgical Technique

In each rat, both sciatic nerves were exposed through a dorsolateral incision and by stomp-cleaving the overlying hamstring muscles [7]. Under the operating microscope, the nerves were dissected free of the surrounding tissue and isolated by a plastic sheet. In the right leg, the sciatic nerve was cut with straight microscissors, and a 5-mm portion of the distal nerve was removed to prevent regrowth of the axons in the distal stump and to achieve the same distance between the nerve stumps as in the laser-operated nerves. In the left leg, the proximal segment of the sciatic nerve was first coagulated over a distance of 7 mm, using an Nd:YAG laser followed by transections of the nerve distally again by the Nd:YAG laser (power 12 W, exposure time several seconds). By vaporizing and coagulating the nerve, a similar distance between the proximal and distal nerve stump was achieved in both legs. After the procedure, the fascia of the hamstring muscles was closed with one or two 6-0 absorbable Dexon sutures, and the skin was closed with 4-0 Dexon sutures (Davis-Geck, Hampshire, UK). The experimental set-up is demonstrated in Figure 1.

Laser System

The laser light source was a continuous-wave Nd:YAG laser (Model CL 60, SLT, Malvern, PA), which emits infrared electromagnetic radiation at a wavelength of 1,064 nm. The light from the laser was delivered to the tissue through a 600-μm-diameter quartz optical fiber with the cladding stripped back for several millimeters. The laser output power was measured before and after laser irradiation with an internal power meter.

Histology

The rats were killed acute ($n = 1$), 30 min ($n = 1$), 3 days ($n = 1$), 1 week ($n = 1$), 3 weeks ($n = 2$), 6 weeks ($n = 2$), and 9 weeks ($n = 6$) after surgery by an overdose of nembutal intraperitoneally, and the nerves including the neuromas were carefully exposed and analyzed. Attention was paid to the size of the neuroma, the adhesions, and the outgrowth of nervous tissue into the distal stump. After dissection, the nerves including the distal nerve stump were fixed in Karnovsky's fixative, postfixed in osmium tetroxide 1%, stained with uranyl acetate, dehydrated in acidified 2,2-dimethoxypropane, and embedded in Epon. After hardening, semithin cross sections were cut (1.25 μm) and stained with toluidine blue and basic fuschine. For electron microscopy, thin sections (70 nm) were cut, stained with tannic acid, uranyl acetate, and lead citrate, and examined in a transmission electron microscope (Philips EM 420, Eindhoven, The Netherlands).

RESULTS

During laser irradiation, whitening and shrinkage of the nerve was observed, while transection of the nerve by laser energy resulted in carbonization at the nerve ends. Microscissor transection resulted in a direct mushrooming of the nervous tissue out of the proximal stump.

There was no postoperative mortality. One rat developed infection of the wound on the left side (laser group), affecting the nerve and sur-

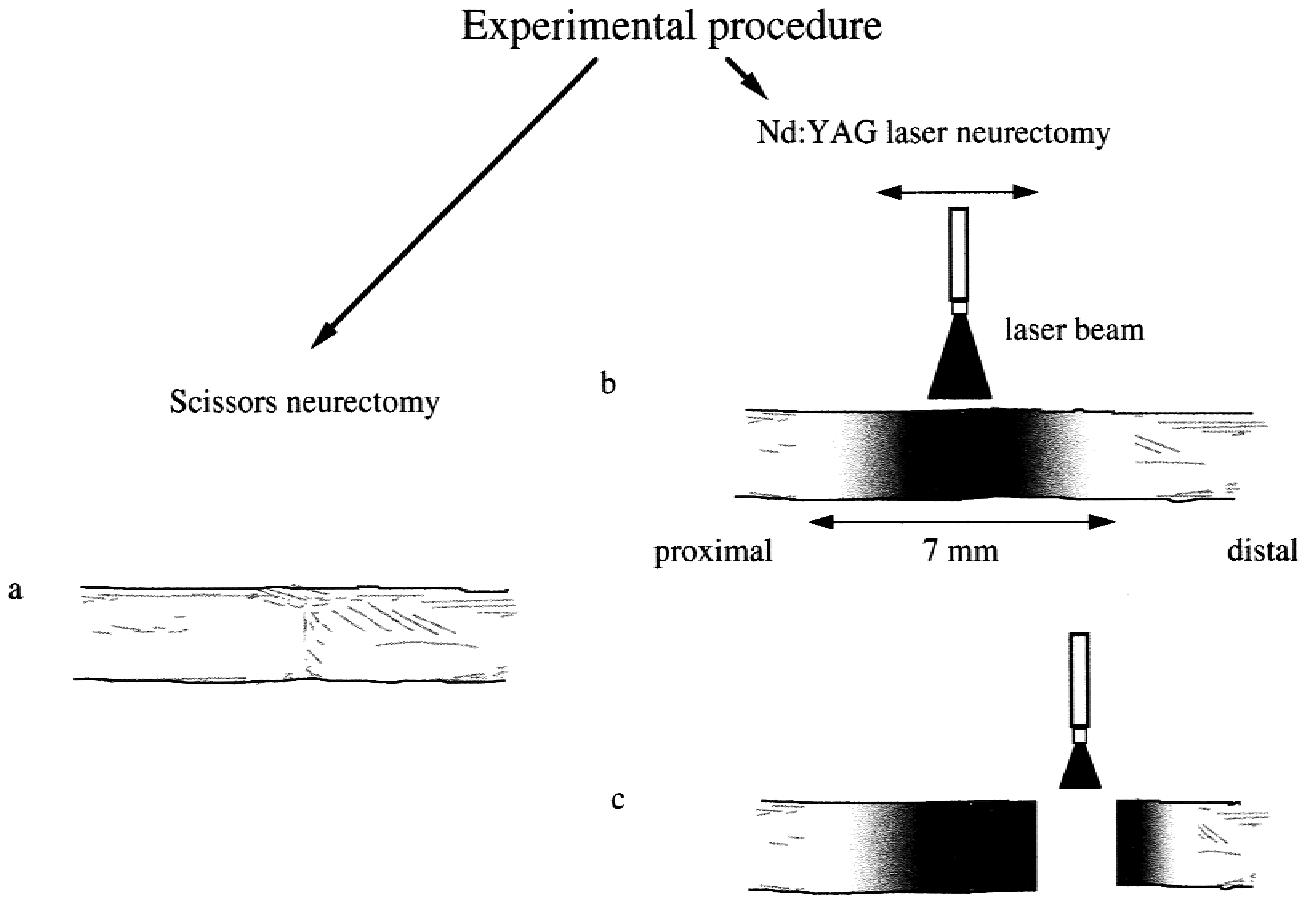

Fig. 1. Schematic drawing of the experimental setup. **a:** Control group. The nerve just before neurectomy with microscissors. **b:** Experimental group. Neurectomy using a modified Nd:YAG laser, in which first a ±7-mm proximal segment of the nerve is coagulated (b), followed by vaporizing of the nerve at the transection site (**c**).

rounding muscles. This nerve was excluded from further study. Automutulation of the hindlegs was present in three rats from the laser group, and four rats in the microscissors group (Table 1).

Gross Anatomy

In the laser-operated nerves, the proximal nerve segments were discolored yellowish, and at the site of transection small black particles were present. In five nerves slight neuromatous swelling was observed with minimal adhesions. The rest of the nerves exhibited no swelling. In all but one nerve there were slight adhesions, with no outgrowth of nervous tissue outside the nerve stump.

Transection with microscissors resulted in widespread neuromas, with a size ranging between approximately double to more than double the size of the proximal portion. The neuromas were characterized by moderate to severe adhesions to surrounding tissue, and in several specimens a slight cylinder of nervous tissue was regenerating into the distal nerve stump. For a summary of the macroscopic data, see Table 1.

Light and Transmission Electron Microscopy

In the first 2 weeks after surgery, laser-transected nerves showed degenerative changes of the axons and myelin sheaths along the coagulated nerve stump. After 3 weeks, axons showed reduced growth within the empty spaces of the proximal segment, and no true neuromas were observed. At the ends of the nerves, carbon particles were observed which were ensheathed by giant cells forming a granuloma. No outgrowth of axons could be observed outside the coagulated proximal stump, and an epi/perineurial layer was covering the nerve stumps. On transmission electron microscopy, the nerve fibers had a specific appearance in which the tissue structure was recognizable but the axons and myelin sheaths were totally thermally degenerated (Fig. 2). Schwann-

TABLE 1. Automutulation, Neuroma Size, and Adhesions Following Laser and Microscissor Neurectomy

Survival time	Rat no.	Automutulation[a]		Neuroma size[b]		Adhesion[c]	
		Laser	Scissors	Laser	Scissors	Laser	Scissors
30 minutes	1	–	–	–	–	–	–
3 days	1	–	–	–	–	–	–
1 week	1	–	–	–	–	–	+
3 weeks	1	1	+++	∞[d]	+++[e]	∞[d]	++
	2	++	+++	+	++	+	+++
6 weeks	1	–	–	+	+++[e]	+	++
	2	–	+	–	++[e]	+	++
9 weeks	1	–	–	+	++[e]	+	++
	2	++	–	–	++[e]	+	++
	3	++	+++	–	+++[e]	+	++
	4	–	–	+	++	++	++
	5	–	–	+	++[e]	+	++
	6	–	–	–	+++[e]	–	+++

[a]Automutulation: –, none; +, slight; ++, moderate; +++, severe.
[b]Size of the neuroma: –, none; +, slight; ++, approximately double the size of the proximal portion; +++, more than double the size of the proximal portion.
[c]Adhesion: –, none; +, slight; ++, moderate; +++, severe.
[d]∞, excluded from the study due to infection of the wound.
[e]Outgrowth of the proximal neuroma into the distal stump.

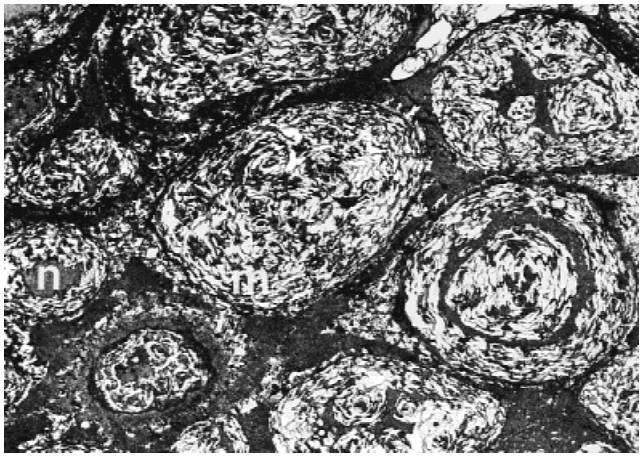

Fig. 2. Transmission electron micrograph of Nd:YAG-transected nerve at 1 week, showing thermally degenerated cellular structure of the axons and myelin sheaths. n, nerve fiber; m, degenerated myelin sheaths which have fallen off in several laminea (original magnification ×1,450).

cell proliferation could not be observed along the coagulated nerve.

Microscissor-transected nerves showed classical neuromas consisting of numerous minifascicles embedded in connective tissue and growing in all directions. Proliferation of fibroblasts and Schwann cells was observed. Some of the regenerating nerve fibers had even escaped from this neuroma and wandered through the intervening scar tissue to enter the distal nerve stump. Small groups of regenerating axons had also penetrated the fibrous covering of the neuroma and had entered the surrounding adhesions.

DISCUSSION

Traumatic transection of a peripheral nerve is inherently associated with the development of posttraumatic neuroma on the end of the proximal stump. After exiting from the edematous proximal stump, the regenerating axons lose their proximal-distal orientations, if a guide structure is not available. They get lost in the scar tissue and grow in a tangle around the proximal stump. Thus, in conjunction with scar tissue a firm tumor, the scar neuroma is formed. This neuroma is characterized histologically by numerous minifascicles, growing in all directions, containing only few or a small group of collateral axonal sprouts encased by a double- or triple-layer perineural covering [1]. Neuromas can be very painful at exposed sites which may arise spontaneously or after mechanical irritation, particularly after amputations [2,8]. Frequently, satisfactory relief does not come from simple resection, since a new neuroma often forms. Among many, often unsuccessful methods of treating stumps of dissected nerves in order to avoid the development of neuromas, heat in the form of electrocautery or laser has been advocated [3,4,6,9–12]. The philosophy behind this treatment lies in the belief that after coagulating or sealing the endoneurial tubules, neuronal outgrowth is prevented.

Three conditions contribute to the formation of a neuroma after transection, i.e., proliferation of Schwann cells in the proximal and distal nerve stump, damaged perineurium, and open endoneu-

rial tubules which facilitate axonal outgrowth. All three adverse events can be successfully avoided by use of the Nd:YAG laser. Firstly, it appears that Schwann cells proliferate as a reaction to the trauma and provide an as yet unidentified trophic substance for the growing and thus regenerating tips of axons [13,14]. In other words, Schwann cells appear to stimulate axonal growth. We have shown that Schwann-cell proliferation is prevented over a considerable distance by laser application, most likely due to thermal injury of the cells. Secondly, although regenerating axons may force their way through dense fibrous tissue, a normal perineurium presents an impenetrable barrier to the passage of axons. Procedures for neuroma prevention will succeed only if a normal perineurium is covering the nerve end before the regenerating axons start to penetrate into the neighboring connective tissue, where their disorderly growth is responsible for the formation of the neuroma. In this context, the Nd:YAG laser is beneficial, as it coagulates and seals the endoneurial tubules and allows the perineurium to grow over the nerve stump before the axons reach the site of transections. Thirdly, the alleged efficacy of the Nd:YAG laser technique is based on the finding that protein synthesis of the nerve cell is regulated by intraperineurial pressure, with the aid of axoplasm flow. As the axons advancing proximally try to enter the coagulated endoneurial tubules, the intraperineurial pressure increases and axoplasm is reduced, thereby inhibiting nerve-cell growth and neuroma formation [15]. In our study, axons exhibited reduced growth within the empty spaces of the coagulated nerve stump, thus limiting uncontrolled growth of axons. This finding is in agreement with Rieske and Kreutzberg [16], who showed that the cell membrane of a neuron is sealed by He-Ne laser (632.8 nm) irradiation, preventing an outflow of cytoplasmic material from the cell.

We believe that the Nd:YAG laser is an excellent tool for preventing neuroma formation, as it has deep optical penetration [17], which allows coagulation of the entire nerve from one-sided irradiation. We chose a power of 12 W, as it was observed in pilot experiments that this power, applied for several seconds, resulted in diffuse thermal coagulation of the rat sciatic nerve. Powers below 10 W produced unequal coagulation within the nerve, while powers higher than 14 W gave rapid vaporization and carbonization. Bipolar electrocautery or the use of a CO_2 laser for coagulation is not suitable, as these instruments will produce an uneven coagulation of the nerve. On the other side, laser wavelengths which give a deep penetration into neural tissue are thus also suitable for this purpose, such as the argon (wavelength of 488–514 nm) and diode lasers (wavelength around 830 nm).

In clinical practice, (recurrent) neuroma formation after neurectomy might be therapy-resistant [1,2]. In such cases, a reoperation offers only a limited number of treatment options. Simple resection of the neuroma results most likely in neuroma formation of the fresh-cut proximal nerve stump. Resection of the neuroma and burying the proximal nerve stump in muscle or bone is a good option for resistant neuromas. Although the numbers were not high enough for significance in this experimental study, we think that the Nd:YAG laser can be safely and effectively applied in the operating room for suppressing neuroma formation. So far, no harmful side effects resulting from the use of the Nd:YAG laser were noted. The primary goal of the treatment is to prevent neuroma development of an already nonfunctioning nerve. Within the limitation of this study it is concluded that the formation of amputation neuromas is suppressed by Nd:YAG laser application.

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